

Ovipositional preference and larval performance of poplar defoliator, *Clostera restituta* on different poplar clones in north-western India

Gurmail Singh • K. S. Sangha

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Abstract: We evaluated ten poplar clones (G-3, G-48, L-50/88, L-154/84, L-156/89, S7C8, S7C15, WSL-22, WSL-29 and Uday) for ovipositional preference and larval performance of *Clostera restituta*. Female moths did not show any preference with respect to clones for oviposition. Significant differences were observed for number of eggs laid on different plant parts. *C. restituta* laid eggs in clusters, preferably on upper surface of leaf and size of the egg cluster varied from 15 to 167 eggs. Clones varied for their relative resistance and susceptibility to *C. restituta*. L-50/88; L-156/89 were identified as most resistant clone based on minimum leaf consumption, whereas S7C15 was found to be most susceptible clone to *C. restituta*. In multiple choice experiments, no feeding preference by *C. restituta* larvae was detected amongst different poplar clones. After initial settlement of larvae on a particular clone, the larvae remained confined to that clone and negligible inter-clonal movement was noticed subsequently. The fresh pupal weight was correlated negatively ($r = -0.37$) with percentage surface leaf area eaten and positively ($r = 0.47$) with length of larval period, measured on different clones. Relationship between percentage leaf area eaten and length of larval period was negative ($r = -0.23$). Owing to relative resistance of L-50/88 and L-156/89 against *C. restituta*, these clones can be recommended for plantation in defoliator prone areas in north-western India.

Keywords: *C. restituta*; feeding; oviposition; *populus deltoides*; preference

Introduction

Poplars are attacked by about 143 species of insect pests in North Western India and about 65 species alone were found to infest *Populus deltoides* (Singh et al. 2004). The indigenous insect fauna recorded on poplar in India chiefly comprises of defoliators (65 spp.), stem and shoot borers (24 spp.) and sap suckers (28 spp.) (Thakur 1999). As the defoliators, the species of genus *Clostera* have been documented in the list of international important pest species. Their distribution extends into all the poplar growing areas in the northern hemisphere: Asia (Wang et al. 1998) besides India; Europe (Allegro 1989) and North America (Ives and Wong 1988). More than 25% defoliation by *Clostera* spp. is known to significantly retard the growth of poplar trees (Gao et al. 1985). Furthermore, artificial defoliation ($> 70\%$) is known to significantly decrease the total biomass of poplar tree by an average of 33% (Reinhenbacker et al. 1996). *Clostera cupreata* (Butler), *Clostera restituta* (Walker) and *Clostera fulgurita* (Walker) often cause large scale defoliation of poplars and are well established on *P. deltoides* in North Western India. Large-scale defoliation of *P. deltoides* by *C. restituta* has been reported from plantations in Satluj river bed areas of Punjab (Sohi et al. 1987). Severe and repeated defoliation of young plants (2–3 yr old) by these defoliators result in their mortality (Singh and Singh 1986). One way to cope with this pest problem is to find resistant/susceptible clones and to manipulate the clones against the pest. Resistance to pests varies among clones, hybrids, cultivars and species of poplar. The variation exists among the poplar clones in terms of growth behaviour of the insects, such as feeding (Robin and Ruffa 1994), feeding potential (Ahmad 1993) and oviposition (Augustin et al. 1993). Oviposition and feeding studies give a fair idea about the performance and preference of the insect species on the host such that clones/ hybrids showing poor performance can be rejected at nursery stage prior to commercial field release. One way to cope with the *C. restituta* is to destroy egg masses. However, the studies related to ovipositional behaviour of the *C. restituta* are still lacking. In this context, the present investigations were carried

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Gurmail Singh

Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India.

K. S. Sangha (✉)

Entomologist in Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana-141004, Punjab, India. Email: kssangha@pau.edu; kamaldeep_sangha@rediffmail.com

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out with the objectives to know the ovipositional preference, ovipositional behaviour and feeding preference of the *C. restituta* among different poplar clones.

Materials and methods

Two experiments were conducted to evaluate ovipositional preference, ovipositional behaviour and feeding preference, of leaf defoliator *C. restituta* on ten different poplar clones from different source/origin (Table 1).

Table 1. Origin/source of poplar clones

Clone	Source and origin	Clone	Source and origin
G-3	Australia	S7C8	Texas, USA
G-48	Australia	S7C15	Texas, USA
L-50/88	USFD*, Lal Kuon, India	WSL-22	WIMCO**, Bagwala India
L-154/84	USFD, Lal Kuon, India	WSL-29	WIMCO, Bagwala India
L-156/89	USFD, Lal Kuon, India	Uday	WIMCO, Bagwala India

*USFD, Uttarakhand State Forest Department; **WIMCO, Western India Match Company

The experiments were carried out at Forest Protection Laboratory and Experimental area of Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana. The test plants were raised and maintained in the field. Cuttings (20-30 cm long and 20-40 mm diameter) of poplar clones were taken from one year old plants and planted in February. Larvae of the defoliator were collected from poplar plantations and culture was maintained in the laboratory for conducting the experiment.

Ovipositional preference

Ovipositional studies were carried out to know ovipositional preference and behaviour of *C. restituta* on different poplar clones. Multiple choice test was conducted under caged conditions (covered with muslin) in the field. Clonal cuttings (WSL-29, L-50/88, L-156/89, S7C8, G-3, G-48, WSL-22, S7C15, L-154/84 and Uday) were planted in Latin square design of 10 × 10 (rows × column), such that each row and column contain each clone once. One such set with hundred plants (ten plants of each clone) was taken as one replication and there were three replications. Fifteen pairs of adults were released in the centre of plants in each replication. After three days of release of the adults all plants were observed for egg laying till all the females were dead. Different plant parts such as upper and lower surface of leaves, stem, petiole and branches were observed to record the number of eggs laid per plant. The data were worked out for the number of egg clusters/plant, number of eggs /clusters, number of eggs/plant and site of oviposition (upper/lower leaf surface, twigs). The data were analysed for analysis of variance (ANOVA) in the Latin square design. The preferred leaf surfaces (upper and lower) for egg laying were analysed by paired t-test.

Larval feeding preference and performance

Feeding preference of *C. restituta* was studied under no choice and multiple choice test conditions during peak activity period of the larvae viz; August, September and October.

No choice tests for larval feeding preference and performance

Pre-measured leaves (cm²) of different clones were placed in Petri-dishes and a single new moulted third instar larva was released in each Petri-dish. There were three replications and five larvae per clone were taken as one replication. The leaves were taken from the middle portion of each clonal plant during August, September and October. After 24 hours of feeding, the leaf area was again measured by leaf area meter and new leaf (pre-measured) was placed in the Petri-dish. Experiment was continued until all the larvae pupated. Larval duration and fresh pupal weight was also recorded. The data were analyzed for mean leaf area (cm²) consumed by the larva per day, weightage percentage of resistance and susceptibility and overall mean leaf area fed. Mean leaf area consumed by larva for each clone was utilized for analysis to test the significance. Weightage percentage of resistance (WPR) was calculated by considering minimum and maximum values of leaf area consumed as 0 and 100 and applying formula

$$WPR = \frac{MaLAF - LAFCC}{MaLAF - MiLAF}$$

where WPR is weightage % of resistance, MaLAF the maximum leaf area fed, LAFCC the leaf area fed (cm) of the concerned clone, and MiLAF is minimum leaf area fed;

$$WPS = 100 - WPR$$

where, WPS is weightage % of Susceptibility

The damage index [based on leaf area consumed (cm²)] was worked out using the formulae given by Ahmad (1993).

$$R1 = \text{Most resistant} = x \leq (\bar{X} - SD)$$

$$R2 = \text{Resistant} = (\bar{X} - SD) < x \leq (\bar{X} + SD/2)$$

$$R3 = \text{Moderately resistant} = (\bar{X} + SD/2) < x \leq \bar{X}$$

$$S1 = \text{Most susceptible} = x \geq (\bar{X} + SD)$$

$$S2 = \text{Susceptible} = (\bar{X} + SD) > x \geq (\bar{X} + SD/2)$$

$$S3 = \text{Moderately susceptible} = (\bar{X}) > x \geq (\bar{X} + SD/2)$$

x = Mean leaf area consumed by larvae for each clone per day
 \bar{X} = Overall mean of leaf area consumed by larvae for all clone per day.

SD = Standard deviation.

Multiple choice tests for feeding preference

Pre-measured leaf discs of different clones were placed at equal distance along the periphery of a plastic tub (20 cm × 10 cm). Twenty new moulted third instar larvae were released in the centre of the tub, covered with muslin and there were three replications in all. Observations were recorded on number of larvae

feeding on each clone after 1, 4 and 24 h of release. Leaf area consumed was determined in each clone after 24 h with the help of leaf area meter. The experiment was carried out during different months. The data were analyzed by completely randomized design.

Results and discussions

Ovipositional preference of *C. restituta*

The study indicated that *C. restituta* females did not show any preference for egg laying on different poplar clones. The mean number of eggs laid per plant ranged from 30 to 167 (Table 2). The observations on the number of eggs laid per clone on different plant parts revealed that the number of eggs laid/clone were statistically non significant. Thus results revealed that there was no antixenosis effect on oviposition preference by *C. restituta*. However, significant differences were found in number of eggs laid on different part of cuttings. Egg laying on muslin cloth covering the sides of the cage, was also observed but that was not taken into consideration for analysis. The maximum mean number of eggs were laid on the upper surface of leaf (65) which was significantly more than lower leaf surface (5.5) ($t_{0.05}$ calculated = 4.23, $t_{0.05}$ table = 1.83). No egg laying was recorded on stem/twig and petiole except G-48 where some eggs were laid on stem. Upper leaf surface was the preferred site for egg laying by *C. restituta*. The laying of few eggs on the stem in G-48 was more an aberration rather than a trend. The eggs were laid in clusters and mean number of eggs per cluster varied from 15 to 167. Mann (1982) reported that *C. restituta* laid eggs in clusters on both the surface of the leaves, but egg cluster on the lower surface was not common. Sangha et al. (2005) also reported that eggs were laid in cluster on both the sides of the leaf and rarely on twigs.

Table 2. Ovipositional preference of *C. restituta* on various plant parts of different poplar clones

Clone	Mean no. of eggs				No. of egg clusters	Mean no. of eggs/cluster
	Per plant	ULS	LLS	Twig		
L-156/89	167	167	0	0	1	167
L-50/88	30	30	0	0	2	15
WSL-29	45	45	0	0	1	45
S7C8	45	45	0	0	1	45
G-3	42	32	10	0	2	21
WSL-22	84	84	0	0	1	84
S7C15	65	65	0	0	1	65
Uday	85	85	0	0	1	85
G-48	97	52	25	20	3	32.33
L-154/84	65	45	20	0	2	32.5
Mean	72.5	65	5.5	-	-	59.18

ULS = Upper leaf surface, LLS = Lower leaf surface, CD(p=0.05)
Clones=Non Significant, $t_{0.05}$ Site (calculated) = 4.23, $t_{0.05}$ (d=9) table = 1.83

Feeding preference of *C. restituta* on different poplar clones

No choice test

The mean leaf area consumed by *C. restituta* varied from 13.03 cm² to 23.53 cm² during August (Table 3). Minimum leaf area consumed on L-50/88 (13.03 cm²) was statistically at par with L-156/89, G-3 and G-48, whereas maximum leaf area was consumed on S7C15 (23.53 cm²) which was at par with L-154/84 and WSL-29. Clones were categorized into five categories as per Ahmad (1993). L-50/88 and L-156/89 were categorized as most resistant (R1), G-3 and G-48 resistant (R2) and WSL-22 and Uday moderately resistant (R3). S7C15, L-154/84 and WSL-29 were categorized most susceptible (S1), whereas S7C8 susceptible (S2). Weightage percentage of resistance (WPR) varied from 63.38 to 100 at the resistant end, whereas weightage percentage of susceptibility (WPS) varied from 65.08 to 100 at the susceptible end during August. Similar results were obtained during September. Leaf consumption was minimum on L-50/88 (10.32 cm²), which was statistically at par with L-156/89, G-3 and G-48, whereas maximum on S7C15 (17.42 cm²) was at par with L-154/84, WSL-29 and S7C8. Weightage percentage of resistance (WPR) varied from 49.66 to 100 at the resistant end, whereas weightage percentage of susceptibility (WPS) varied from 72.75 to 100 at the susceptible end. All the clones were found to fall into same categories as during August. However, during October, maximum and minimum leaf areas consumed were 13.72 cm² and 7.85 cm², respectively. The lowest consumption on L-50/88 (7.85 cm²) was at par with L-156/89, G-3 and G-48, whereas highest on S7C15 (13.72 cm²) was at par with L-154/84 and WSL-29. L-50/88 and L-156/89 were most resistant (R1), G-48 and G-3 resistant (R2) and Uday moderately resistant (R3), whereas three clones (S7C15, L-154/84 and WSL-29) were found most susceptible (S1) and two clones (WSL-22 and S7C8) moderately susceptible (S3). Weightage percentage of resistance (WPR) varied from 52.13 to 100 at the resistant end, whereas weightage percentage of susceptibility (WPS) varied from 58.45 to 100 at the susceptible end. G-48 and G-3 were reported resistant to *C. cupreata* by Singh and Pandey (2000). Ahmad (1993) and Singh (2002) identified G-48 resistant clone against *C. cupreata* too. These results were in accordance to present study. Ahmad (1993) and Singh and Pandey (2000) had reported S7C8 susceptible to *C. cupreata*, which is also in accordance to present study. WSL-22 identified moderately susceptible to *C. cupreata* by Singh (2000), was also found susceptible to *C. restituta*. S7C15 and WSL-29 were identified most susceptible clones in present study, which was in conformity with earlier study by Singh and Pandey (2002). The mean leaf area consumed (per day) by the larvae of *C. restituta* decreased from August to October. This may be due to increase in toughness or change in nutritional composition of the leaves as the plant aged. The results revealed that clones L-50/88 and L-156/89 were relatively resistant to *C. restituta*. These clones may be further evaluated for the mechanism of resistance.

Multiple choice test

No particular trend was observed for larval feeding on leaf discs of various clones during different months (Table 4). Mean number of larvae feeding per clone after 24 h of release varied 1.00–3.12, 1.43–3.00 and 1.50–2.89 during August, September and October month, respectively. Leaf area consumed after 24 h ranged 2.63–4.70, 2.61–4.24, 2.30–3.80 (cm²) during August, September and October, respectively. The difference in leaf con-

sumption was due to the difference in number of larvae feeding on different poplar clones. It was evident from the data, that after initial settlement of larvae on a particular clone, the larvae remained confined to that clone and negligible inter-clonal movement was noticed thereafter. The results showed no significance difference in selection of poplar clones by *C. restituta*. This suggests that relative preference/non preference may be attributed to antibiosis rather than antixenosis. Similar results were obtained by Singh (2004) in case of *C. fulgurita*.

Table 3. Relative larval performance of *C. restituta* on different poplar clones during different months, under no choice conditions

Clone	Mean leaf area consumed (cm ²)*			Weightage percentage of Resistance/Susceptibility			Relative resistance/susceptibility category (as per Ahmad 1993)		
	August	September	October	August	September	October	August	September	October
L-50/88	13.03	10.32	7.85	100	100	100	R1	R1	R1
L-156/89	13.32	11.46	8.62	97.2	84.03	86.76	R1	R1	R1
G-3	14.58	11.75	9.22	85.16	79.88	71	R2	R2	R2
G-48	15.77	11.85	9.55	74.37	78.53	76.55	R2	R2	R2
WSL-22	16.32	13.9	11.51	68.64	49.66	62.37	R3	R3	S3
Uday	16.87	13.41	10.66	63.38	56.53	52.13	R3	R3	R3
S7C15	23.53	17.42	13.72	100	100	100	S1	S1	S1
L-154/84	22.57	17.06	12.75	90.83	94.8	83.48	S1	S1	S2
WSL-29	21.73	16.52	13.2	82.86	87.24	91.21	S1	S1	S1
S7C8	19.86	15.49	11.28	65.08	72.75	58.45	S2	S2	S3
CD (p=0.05)	2.92	2.98	1.89	-	-	-	-	-	-
X [□] (Overall mean)	17.76	13.91	10.84	-	-	-	-	-	-
SD	3.88	2.98	1.91	-	-	-	-	-	-

Table 4. Relative preference of *C. restituta* larvae under multiple choice test conditions

Clones	Mean no. of larvae feeding per clone									Mean leaf area (cm ²) consumed after 24 h		
	August			September			October			Aug	Sept.	Oct.
	1 h	4 h	24 h	1 h	4 h	24 h	1 h	4 h	24 h			
L-50/88	0	1.75	1.27	0	2.22	2.22	0	1.56	1.46	3.42	3.15	2.30
L-156/89	1	2.00	1.12	0	2.74	2.75	1	2.00	2.03	2.63	2.70	2.98
G-3	1	3.15	3.12	2	2.26	2.00	1	2.00	2.17	4.70	3.65	3.40
G-48	0	2.37	2.37	0	1.50	1.50	0	1.25	1.39	3.80	2.87	2.77
Uday	1	1.00	1.00	1	1.48	1.78	1	1.53	2.00	2.68	2.61	2.56
WSL-22	2	1.16	1.70	0	2.02	2.00	1	1.90	1.50	3.85	3.59	3.50
S7C15	1	2.32	2.40	1	3.00	3.00	1	3.00	2.89	4.56	4.24	3.80
L-154/84	1	3.25	3.02	0	1.50	1.93	1	2.69	2.00	3.79	2.86	3.10
WSL-29	1	1.00	2.00	0	1.77	1.43	0	1.56	2.00	3.72	2.76	2.79
S7C8	0	2.00	2.00	1	1.51	1.60	1	2.51	2.56	3.78	2.88	3.29
CD	-	0.23	0.26	-	0.17	0.23	-	0.22	0.23	0.28	0.21	0.23

Correlations analysis

The correlation analysis between three variables [relative (i) pupal fresh weight (ii) leaf area eaten and (iii) the length of larval period] was also carried out to establish/ confirm the link between them. Mean pupal weight of both the sexes of *C. restituta* and percentage leaf area eaten in 24 h were negatively correlated ($r = -0.37$) (Fig. 1). Relationship between percentage leaf area

eaten in 24 h and larval period (3–5th instar) measured on different poplar clones was also negative ($r = -0.23$) (Fig. 2) whereas mean pupal weight (both the sexes) of *C. restituta* and larval period (3–5th instar) were positively correlated ($r = 0.47$) (Fig. 3). The results were in agreement with findings of Singh and Pandey (2002) in case of *C. cupreata*. For lepidopterous defoliators, a slow rate of development and higher female pupal weight are positively correlated with each other (Grayson and Edmunds 1989). In addition, for poplar leaf mining Lepidoptera (*Phylloc-*

nistis unipunctella) the length of larval mines has been found to be negatively correlated with its pupal weight because of lower palatability due to higher tannin content (Nef 1986).

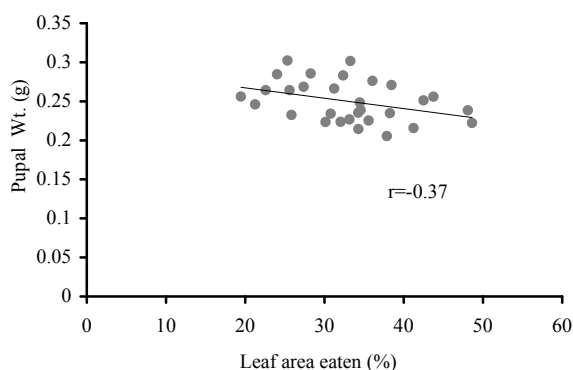


Fig. 1 Relationship between mean pupal weight of both the sexes of *C. restituta* and leaf area eaten measured on different poplar clones.

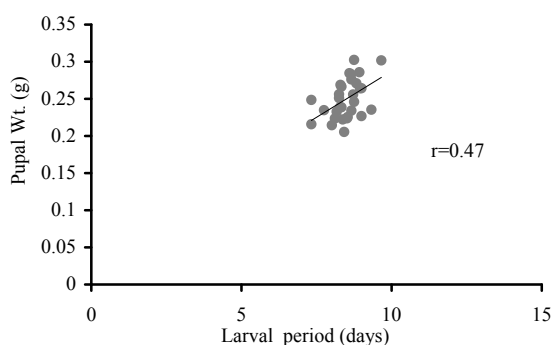


Fig. 2 Relationship between mean pupal weight of both the sexes of *C. restituta* and larval period measured on different poplar clones.

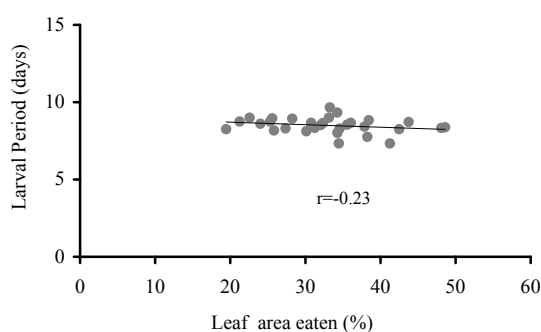


Fig. 3 Relationship between larval period of *C. restituta* and leaf area eaten measured on different poplar clones

Conclusion

C. restituta laid eggs in clusters preferably on upper surface of the leaves but did not showed preference for oviposition among various poplar clones. L-50/88 and L-156/89 were identified as

most resistant clones based on minimum leaf consumption, whereas S7C15 was found to be most susceptible clone to *C. restituta*. Egg mass destruction and planting L-50/88 and L-156/89 clones in defoliation prone areas, should be integrated in to management strategies for poplar defoliators. The role of leaf hairs, antifeedants (tannins, phenolic and glucosides), feeding stimulants, which are known to significantly influence the oviposition, feeding performance and preference of leaf eating insects, need to be examined, for better interpretation of the results.

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